Research Article

Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil

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Abstract

Heavy metals polluted soils have turned out to be a common environmental problem across the globe due to their toxic effects and accumulation through the food chain. Heavy metals have lethal effects on all forms of life. For instance, plants grown on heavy metal polluted soil show a reduction in growth and yields. A surge in anthropogenic activities and industrial operations has substantially increased the level of heavy metal pollution and release into the environment; hence, there is need to remediate these heavy metal pollutants. Biosorption is an efficient, economical, ecofriendly and convenient techniques of remediating heavy metal polluted soils. It is a widely accepted method that utilizes biomaterials such as natural biomass as biosorbents. The current study was based on the biosorption of copper, chromium, cadmium and nickel polluted soil using bacteria and fungi isolated from soil. Bacterial species isolated were *Pseudomonas*, *Bacillus*, *Micrococcus*, *Escherichia*, *Streptococcus*, *Enterobacter* and *Staphylococcus* while fungi isolated were *Aspergillus niger*, *Penicillium notatum* and *Aspergillus flavus*. The isolated bacteria were screened for potential to biosorb copper and chromium likewise fungi for cadmium and nickel. Biosorption rate was determined using atomic absorption spectrophotometry. Five milliliters each of a-day-old culture of the screened bacteria and fungi was inoculated into 45 ml of nutrient broth (bacteria) and potato dextrose broth (fungi) having concentrations of 5, 10, 15 and 20 ppm, respectively, of copper, chromium, cadmium and nickel. The conical flasks were incubated at a temperature of 37 °C and 28 °C ± 2 for bacteria and fungi, respectively, for a period of 35 days of inoculation. For the bacterial isolates, the highest biosorption rates of chromium (89.67%) and copper (90.89%) by *Pseudomonas aeruginosa* were observed at 20 ppm on day 21 and 15 ppm on day 14, respectively, while for the fungi isolates, *P. notatum* showed highest biosorption rate for cadmium at 10 ppm with 77.67%. *Aspergillus niger* showed highest biosorption rate for nickel with 81.07% after 28 days of incubation. The results of this study revealed the ability of *Pseudomonas aeruginosa* to biosorb copper and chromium and also *A. niger* and *P. notatum* to biosorb cadmium and nickel from the environment and can be developed for the biosorption of soils polluted with copper, chromium, cadmium and nickel.

Keywords Biosorption · Cadmium · Copper · Nickel · Chromium · Bacteria · Fungi

1 Introduction

Heavy metals are natural elements with atomic number greater than 20, characterized by a relatively high density (at least 5 g cm^{-3}), and are toxic even at low concentrations [1–3]. They are characteristically existing components found in changing variation in the environments and are part of human daily activities, they are also found in important structures and in a range of other artificial mixes [4]. The activities of human have greatly impacted on some heavy metal biochemical cycles and equalization of which

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SN Applied Sciences (2019) 1:857 | https://doi.org/10.1007/s42452-019-0879-4

Received: 11 April 2019 / Accepted: 2 July 2019 / Published online: 15 July 2019

a great number of heavy metals have found its use in various items such as cars and batteries [5].

Heavy metals are generated from both anthropogenic and natural sources and are eventually discharged into the environment [6, 7]. The main natural discharge of heavy metals is during volcanic eruptions and weathering of metal-bearing rocks [2]. The discharge of heavy metals through various man-made activities, for example excessive application of chemical fertilizers, wood burning, coal combustion, vehicle exhaust, mining, smelting and incineration [2], has caused a wide spread disruption of the normal biogeochemical cycles of metals causing a larger accumulation of heavy metals in the environment, especially the soil [6–8]. The major heavy metals of concern include lead, cadmium, arsenic, mercury, copper and chromium because of their toxic impact on human health; for instance, environmental exposure to high concentrations of heavy metals has been linked with various cancers and kidney issues [9]. Heavy metals have also greatly affected soil microorganisms and plants growth and development [10]. The presence of heavy metals in the environment has been a source of concern over the past few decades due to their persistence, potential harm and toxicological hazards [2]. Besides the fact that they are non-biodegradable, they may also undergo microbial or chemical transformation [8, 9, 11, 12]. Recently, Hasani et al. [13] and Nath et al. [3] reported that heavy metal polluted environments activate co-selection process and cause a decrease in microbial tolerance to antibiotics due to their ability to co-regulate genes responsible for antibiotic resistance.

The soil is a reservoir for some essential trace elements such as zinc and copper, which are necessary for the growth of plants and animals, but external influence can increase their concentration and consequently reduce the overall soil fertility and agricultural productivity. Therefore at soil concentration above normal level, if permitted to accumulate in the food chain, heavy metals such as lead and cadmium can have adverse effects on human and animal health [14]. There is also the risk of leaching of heavy metals, and this may contaminate underground water and in turn affect human health, especially those that consume underground water through boreholes and well water [1]. The increase in industrialization and urbanization offers ascend to heavy metal pollution of the environment, which might have resulted from the discharge of effluents containing metals such as lead, cadmium, chromium, nickel and mercury [6, 7, 15].

According to Thompson and Darwish [14], heavy metals are of genuine environmental concern because of their potential toxicity, reactivity and soil mobility. The emissions of these metal pollutants have become a severe threat to mankind. The routes of exposure of human to heavy metals include inhalation, dermal absorption and ingestion [2, 16]. In order to alleviate the environmental impacts of heavy metal, several efforts are currently being adopted. Such methods include thermal, chelating, precipitation, adsorption, ion exchange, membrane technologies and biosorption strategies. Biosorption has several advantages over other conventional methods of heavy metal remediation because of its accessibility and efficiency [15]. One important economic aspect of biosorption technology is that the biomass used for decontamination of heavy metal pollutants is natural, easily available and affordable, and also it provides a better performance compared to conventional methods of decontamination [17, 18]. Thus, there is the need to apply affordable metal remediation technology like biological method so as to reduce the toxic effect of these heavy metals in the environment.

Biosorption is a biological remediation technology that involves the removal of metal species from a solution by inexpensive biomaterials, and it has been reported that most biological materials can be useful as biosorbents for heavy metals sequestration and can be a vital passive procedure in organisms, but the exceptions are mobile alkali metal cations like Na^+ and K^+ [19]. Most biosorbent materials have good biosorption capacities toward all types of metal ions, so many affordable and easily available biosorbents used for the elimination of heavy metals in the environment are mainly derived from bacteria, fungi, algae, plants and some polysaccharide materials. Many researches involving biosorption of heavy metals from the environment have been carried out in vitro and in vivo [19]. Karthik et al. [20] and Karthik et al. [21] reported the biosorption and bioaccumulation of high chromium by *Cellulosimicrobium funkei* AR8 and AR6, respectively, under batch conditions. Dhanarani et al. [15] reported the biosorption of aluminum by *Bacillus safensis*. Live and dead biomass of *Aspergillus niger* have been used to biosorb fluoride in aqueous solution under batch and continuous condition [22].

The materials used for biosorption include a solid stage biomaterial (sorbent) and a solvent stage containing disintegrated species like metal particles to be sorbed (sorbate). Similar to each sorption procedure, binding of sorbate species to biosorbent proceeds until it reaches a balance between the sorbate species in fluid and solid stages. Biosorbents contain some atomic groups that have tendency to sorbates, for example, metal ions. This innovation utilizes different sorts of biomass to expel heavy metals from contaminated environment [19, 23].

Biosorbents of biological origin particularly various microorganisms have received growing interest for the removal of heavy metal and recovery owing to their greater performance [7, 24]. Although biosorption is influenced by many factors such as pH, temperature and contact time [6, 25], the use of microorganisms as biosorbent

materials offers a selective removal of heavy metals under varied physicochemical properties, adsorption and desorption and another advantage of microorganisms is their high surface-to-volume ratio [7, 17]. Several microorganisms have been used for biosorption, examples include *Bacillus cereus* used in Cd [26] and Cu [6] removal, *Cellulosimicrobium funkei* AR6 and *Cellulosimicrobium funkei* AR8 used in biosorption of Cr [20, 21], *Bacillus safensis* used to biosorb aluminum [15], *Aspergillus niger* used for fluoride biosorption [22], and Pugazhendhi et al. [6] reported lead biosorption using *Ralstonia solanacearum*. Therefore, this study is aimed at determining the biosorption rate of cadmium, nickel, chromium and copper by fungi and bacteria isolated from soil.

2 Materials and methods

2.1 Collection of soil samples

Soil used was collected from botanical garden of Biological Science Department, Federal University of Technology, Minna, Nigeria, and transported in a polythene bag to the microbiology laboratory for further analysis.

2.2 Media preparation

For fungi isolation, potato dextrose agar was prepared according to manufacturer's instruction and was autoclaved at 121 °C for 15 min and was allowed to come to room temperature, and then, chloramphenicol would be added to the media to inhibit bacteria growth while for bacteria isolation, nutrient agar and nutrient broth were prepared according to manufacturer's specification and were autoclaved at 121 °C for 15 min and then aseptically transferred into petri dishes and conical flask, respectively, and allowed to solidify.

2.3 Isolation of microorganism

The microorganisms were isolated by pour plate technique on potato dextrose agar (PDA) for fungi and nutrient agar (NA) for the enumeration of aerobic heterotrophic bacteria. Prior to isolation, the soil samples were serially diluted from 10^{-1} to 10^{-10}. The 1 ml of the diluents from 10^{-4} and 10^{-6} was aseptically inoculated into media using pour plate method and incubated at ambient temperature for 24–72 h for fungi and 37 °C for 24 h for bacteria. Each colony that appeared on the plate was considered as one colony-forming unit (cfu). The bacterial and fungal colonies were subcultured repeatedly on potato dextrose agar and nutrient agar plates, respectively, to obtain a pure isolate. The pure isolates were stored in agar slants for further characterization and identification [27].

2.4 Identification of fungi isolates

After obtaining a pure culture of the fungal isolates, macroscopic and microscopic examination of pure isolates was carried out. The characterization was based on the colonial and morphological characteristics. The fungal colony was observed, and microscopic examination was carried out by placing a drop of distilled water and a portion of the fungi on a slide, covered with a cover slip and observed on a microscope with 10× objective and then 40× objective. Important details such as vegetative structure of hypha, septa, etc., as well as reproductive structures including the type and shape of spore were noted for identification of each isolate [28].

2.5 Characterization and identification of bacterial isolates

The bacterial isolates were characterized by colonial morphology and biochemical characteristics such as Gram stain, spore stain, motility, catalase, oxidase, coagulase, indole, methyl-red test (MR-VP), urease, Simmons citrate test and triple sugar iron agar using methods described by Cheesbrough [27] and Bergey's manual of determinative bacteriology [29].

2.6 Preparation of metal solutions

The stock solution of cadmium sulfate and nickel sulfate was prepared by dissolving 3.73 g and 4.48 g, respectively, in 1 L of distilled water. Also, the stock solution of potassium dichromate ($K_2Cr_2O_7$) and copper sulfate ($CuSO_4$) was prepared by dissolving 2.5 g and 5.8 g, respectively, in 1 L of distilled water. The stock solutions were agitated for 15 min and then allowed to stand for a period of 24 h in other to obtain a complete dissolution of salt. The initial cadmium, nickel, copper and chromium concentrations were measured using atomic absorption spectrophotometry (UV–Vis 752, UK). The pH of the solution was also adjusted to pH 7 using sodium hydroxide (NaOH) and hydrochloric acid [18].

2.7 Screening of microorganisms for biosorption of heavy metals

The isolates were randomly screened for their abilities to biosorb the heavy metals. The 5 ml of fungi culture was inoculated into 45 ml of potato dextrose broth having 5 ppm of Cd and Ni separately. Similarly, 5 ml of a-day-old bacterial culture was inoculated into 45 ml of nutrient

broth having 5 ppm of Cu and Cr separately. The metal pH solution was adjusted to the pH value of 7 before the different isolates were added to the solution. The conical flasks containing PDA were incubated at 28 ± 2 °C while those containing nutrient broth were incubated at a temperature of 37 °C. Each conical flask was withdrawn after 7 days of inoculation; centrifugation was done at 1792 G for 25 min. The supernatant was digested using nitric acid of 4 ml for every metal solution sample. The concentration of metal was determined by absorption spectrophotometry (UV–Vis 752, UK) [17]. The percentage of biosorption was determined by Beer Lambart's law: (%) biosorption = initial metal concentration – final metal concentration*100/initial metal concentration.

2.8 Biosorption of heavy metal

Five milliliters (5 ml) broth of fungi culture was inoculated into 45 ml of potato dextrose broth having different concentration (5, 10, 15 and 20 ppm) of Cd and Ni separately. Five milliliters (5 ml) of a-day-old bacterial culture was inoculated into 45 ml of nutrient broth having different concentrations of Cu and Cr separately (5 ppm, 10 ppm, 15 ppm, 20 ppm). The metal pH solution was adjusted to the pH value of 7 before the different isolates were added to the solution. The conical flasks containing PDA were incubated at 28 ± 2 °C while those containing nutrient broth were incubated at a temperature of 37 °C. Each conical flask was withdrawn at specific time intervals of 7, 14, 21 and 28 days of inoculation, and centrifugation was done at 4000 rpm for 25 min. After centrifuging, the supernatant was digested in correspondence with their varying concentration using nitric acid of 4 ml for every metal solution sample. The concentration of metal was determined by absorption spectrophotometry (UV–Vis 752, UK) [17]. The percentage of biosorption was determined by Beer Lambart's law: (%) biosorption = initial metal concentration – final metal concentration * 100/initial metal concentration.

2.9 Data analysis

Data generated from this study were subjected to statistical package for social science (SPSS 23) using one-way analysis of variance (ANOVA) and Tukey's HSD.

3 Results

3.1 Microorganisms isolated from the soil samples

Seven bacterial isolates were obtained after series of subculturing of the bacterial cultures isolated from the soil. The bacteria were identified as species of *Bacillus*, *Escherichia*, *Micrococcus*, *Enterobacter*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* while the fungi isolates were identified as *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*.

3.2 Microorganisms screened for biosorption potential

Screening of fungi isolate for biosorption potential shows that the three fungi were capable of absorbing the heavy metals. *Aspergillus niger* showed a higher sorption rate of 43.69% in nickel solution, *Penicillium notatum* also showed a high sorption rate of 38.87% in cadmium solution while *Aspergillus flavus* gave a lower biosorption rate of 42.69% in cadmium and 17.0% in nickel solutions of the same concentration and at the same time intervals. There was a significant difference ($P < 0.05$) between the % biosorption rates by each isolate (Table 1).

The bacterial isolates were subjected to screening under similar metal concentration and environmental conditions to check for their potential to carry out the biosorption process. After subjecting the isolated bacteria to screening, all the bacteria isolated were capable of biosorbing chromium and copper but at different rates. *Pseudomonas aeruginosa* showed the highest biosorption rate and was observed to be more effective compared to the other isolated bacteria. There was a significant difference ($P < 0.05$) between the % biosorption rates by each isolate (Table 2). Based on these results, *Aspergillus niger* was used to biosorb nickel, *Penicillium notatum* was used to biosorb cadmium while *P. aeruginosa* was utilized in the biosorption of copper and chromium.

3.3 Biosorption of cadmium and nickel by fungi isolates

The biosorption rates for *Penicillium notatum* and *Aspergillus niger* are shown in Figs. 1 and 2, respectively. The highest biosorption by *Penicillium notatum* was observed

Table 1 Fungi isolates screened for biosorption potential

Fungal isolates	Cadmium (%)	Nickel (%)
Aspergillus niger	19.18[b]	**43.69**[a]
Aspergillus flavus	17.00[c]	42.69[b]
Penicillium notatum	**38.81**[a]	20.00[c]
Control	1.84[d]	1.65[d]

Mean of values in the same column with different superscripts differs significantly ($P < 0.05$) from each other

Bold indicates the highest values obtained during the screening for the ability of the microorganisms to utlise the heavy metals. That informed their selection for the biosorption experiments

Table 2 Bacteria isolates screened for biosorption potential

Bacterial isolates	Chromium (%)	Copper (%)
Bacillus lentus	38.03[b]	36.33[b]
Escherichia coli	4.47[g]	8.63[f]
Micrococcus roseus	36.56[c]	20.33[c]
Enterobacter aerogenes	22.09[d]	14.87[e]
Pseudomonas aeruginosa	**45.59**[a]	**42.69**[a]
Staphylococcus aureus	19.94[e]	17.28[d]
Streptococcus species	13.87[f]	4.86[g]
Control	0.41[h]	0.65[h]

Mean of values in the same column with different superscripts differs significantly ($P < 0.05$) from each other

Bold indicates the highest values obtained during the screening for the ability of the microorganisms to utlise the heavy metals. That informed their selection for the biosorption experiments

at 20 ppm with 77.67% on the 28th day, and the lowest was observed at 10 ppm on the 7th day (Fig. 1). At day 7 of 5 ppm, 10 ppm, 15 ppm and 20 ppm, lower biosorption rates were observed by *Aspergillus niger* with 43.69%, 26.04%, 42.17% and 56.98% respectively while at the end of 28th day higher sorption rate of 75.46%, 72.79%, 80.11% and 81.07% were recorded for 5 ppm, 10 ppm, 15 ppm and 20 ppm, respectively (Fig. 2).

3.4 Biosorption of chromium and copper by bacterial isolates

The bacterial isolates were subjected to screening under similar metal concentration and environmental conditions to check for their potential to carry out the biosorption process. After subjecting the isolated bacteria to screening, all the bacteria isolated are capable of biosorbing heavy metals but at different rates. *Pseudomonas aeruginosa* showed highest biosorption rate and was observed to be more effective compared to the other isolated bacteria and the control. The biosorption rates generally increased, corresponding to increase in the days of

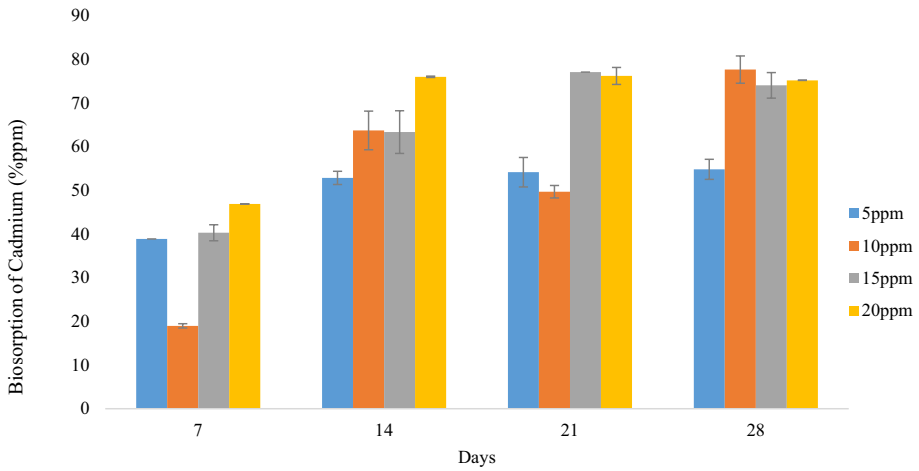

Fig. 1 Biosorption of cadmium by *Penicillium notatum*

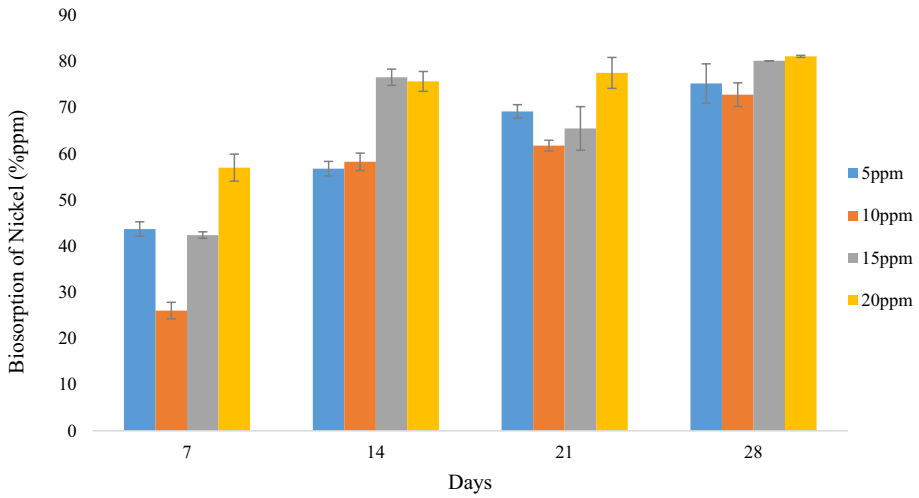

Fig. 2 Biosorption of nickel by *Aspergillus niger*

incubation. The highest rate was observed at 20 ppm after 21 days (Fig. 3) and 15 ppm after 14 days (Fig. 4).

4 Discussion

The result of screening of fungi isolate for biosorption potentials revealed that of the three different species of fungi isolated, *Aspergillus niger* showed a significantly higher biosorption rate of 43.69% in nickel solution ($P < 0.05$) while *Penicillium notatum* also showed a significantly higher sorption rate of 38.87% in cadmium solution ($P < 0.05$). The ability of these isolates to take up the heavy metals may be due to some of their inherent physiological characteristics such as the cell wall. *Penicillium notatum* has a rigid and complex cell wall that contains polysaccharides such as chitins and glucans, and also has higher surface-to-volume ratio which help these fungi to absorb cadmium into their cell wall [30].

Penicillium notatum also releases some extracellular enzymes such as laccases and metal binding proteins that act as chelators that binds heavy metals and facilitates absorption by the cell wall. When compared to control, a high level of cadmium was taken up by *Penicillium notatum* in that solution. Similar findings were recorded by Leitao [31] who reported that *Penicillium notatum* isolated from heavily polluted streams near industrial area was able to grow and remove 100-fold higher cadmium level after 13 days of incubation by an absorption process. Also Abdulwahab [32] reported that *Penicillium notatum* can survive in a mineral liquid medium containing up to 400 µg/ml of cadmium and other metals such as zinc, aluminum and zinc.

Similarly, *Aspergillus niger* is a filamentous fungus that is capable of absorbing nickel from the environment. Just like *Penicillium notatum*, the metal binding capacity of *Aspergillus niger* is due to its physiological characteristics. *Aspergillus niger* has a complex cell wall that is made up of chitins, glucans, inorganic ions, lipids, nitrogen

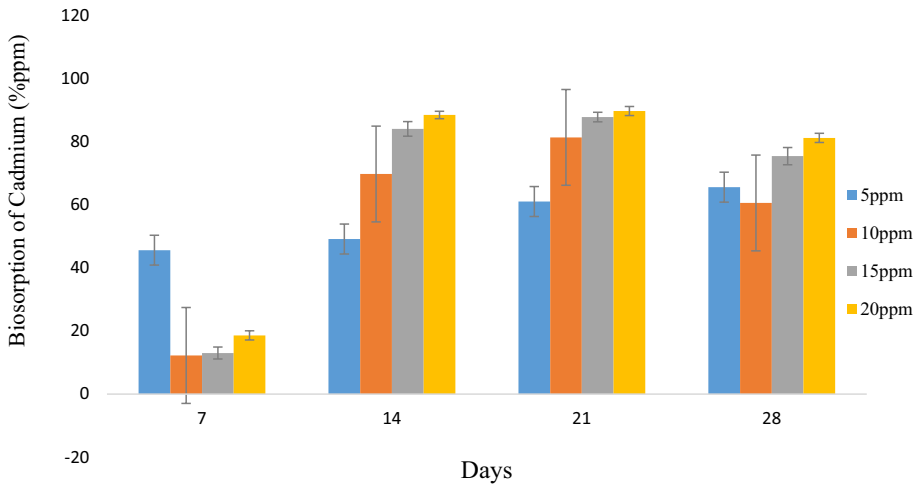

Fig. 3 Biosorption of chromium by *Pseudomonas aeruginosa*

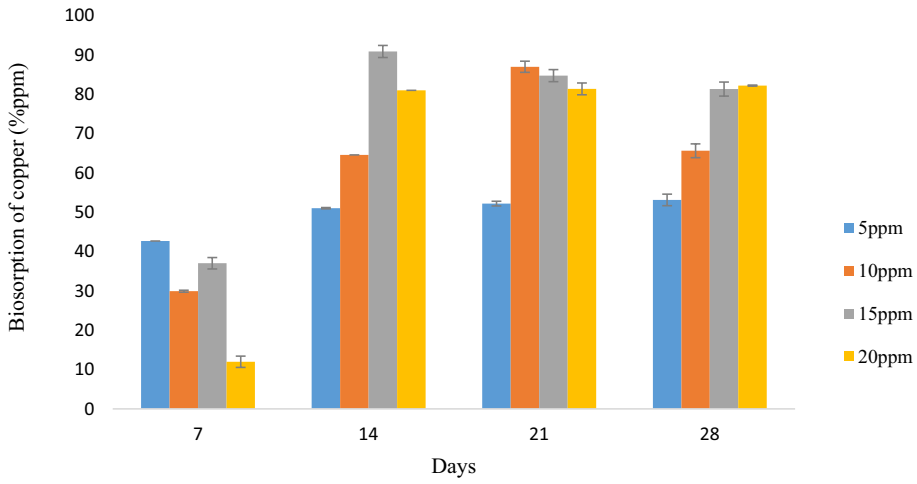

Fig. 4 Biosorption of copper by *Pseudomonas aeruginosa*

containing polysaccharide and proteins that can tolerate and detoxify nickel by active uptake [30]. Extracellular and intracellular precipitation of these features allows *Aspergillus niger* to absorb nickel into their cell wall [30]. Compared to control, high level of nickel was taken up by *A. niger* in that solution (Fig. 2) which shows that *Aspergillus niger* is capable of absorbing nickel in a solution. The highest sorption rate observed on the 21st day at 15 ppm and 20 ppm and not on the 28th day may be due to the fact that cadmium is toxic to the fungal cells at higher concentration and longer period of exposure could damage the cells of these fungi, which may lead to their death and reduce the biosorption rate. This result is similar to the result obtained by Iqbal et al. [33] who isolated *Aspergillus niger* and *Penicilluim* species from soil for biosorption, and recorded that *Aspergillus niger* and *Penicillium* species have promising biosorption capacity for nickel, chromium and cadmium in solution, but *Aspergillus niger* shows preference to nickel compared to *Penicillium* species.

The use of *Pseudomonas* species was studied in different concentrations of chromium (5, 10, 15 and 20 ppm) and also at different time intervals (7, 14, 21 and 28 days). The highest biosorption rate of chromium by *Pseudomonas aeruginosa* was observed at 20 ppm on day 21 (Fig. 3), and the highest biosorption rate of copper by *Pseudomonas aeruginosa* was recorded at 15 ppm on day 14 (Fig. 4). The ability of *Pseudomonas aeruginosa* to take up certain heavy metal from the environment may be due to the nature of their cell wall. The cell wall of *Pseudomonas* species contains lipopolysaccharides, protein and phospholipids. The lipopolysaccharide present in the cell wall contains phosphate and carboxyl groups and phospholides, which are the primary sites of metal ion binding, thereby making biosorption process possible by the microorganism. Abioye et al. [23] found that *Pseudomonas aeruginosa* has been accounted for as effective chromium reducer. In comparative study, on the selective binding of different metals to the cell wall of *Pseudomonas* species, copper had much more affinity than other heavy metals like nickel and cadmium when evaluated together [34, 35].

There was an increased biosorption rates on day 7 down to day 21 in 5, 10, 15 and 20 ppm concentration of chromium and copper. After day 21, there was a slight decline in sorption by the organism. The reason for the decline in the rate of biosorption from day 21 and 28 of chromium and copper could be as a result of the saturation of the organism-metal binding sites or could be as a result of the fact that chromium and copper at higher concentration and a longer period could cause damage to the cell of the microorganism.

5 Conclusion

The study was based on the biosorption of copper, chromium, cadmium and nickel polluted soil using *P. aeruginosa*, *Aspergillus niger* and *Penicillium notatum* isolated from soil. The results obtained from this study revealed that the isolates were able to biosorb various concentrations of the heavy metals and can be developed for the biosorption of soils contaminated with copper, chromium, cadmium or nickel.

Compliance with ethical standards

Conflict of interest There is no conflict of interest in the preparation of this article.

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